

**REMARKS**

***Status of the Claims***

Claims 12-22 and 27-38 are in the application.

Claims 12-22 and 27-38 were rejected.

By way of this amendment claims 12-18, 27, 28, 31 and 34 have been canceled, claims 19, 22, 29, 32, 33, 35 and 38 have been amended, and new claims 39-48 have been added.

Upon entry of this amendment, claims 19-22, 29, 30, 32, 33 and 35-48 will be pending.

***Summary of the Objections/Rejections***

Objections have been made to claims 14-19, 22, 34 and 35.

Claims 12-22, 27 and 28 have been rejected under 35 U.S.C. 112, second paragraph, as failing to particularly point out and distinctly claim subject matter which Applicant regards as the invention.

Claims 12-18, 22, 31 and 32 have been rejected under 35 U.S.C. 101 as being ineligible subject matter.

Claims 12-18, 27-30 and 33-38 have been rejected under 35 U.S.C. 102(a) as being anticipated by Doench et al.

Claims 12, 14-18, 27 and 28-30 have been rejected under 35 U.S.C. 102(b) as being anticipated by Zeng et al.

Claims 12-17 and 31 have been rejected under 35 U.S.C. 102(e) as being anticipated by Bentwich et al. in view of evidence in Tinoco.

Claims 12-22, 27, 29-31 and 33-37 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Bentwich.

Accordingly, the only rejection of claim 32 is under 35 U.S.C. 101, the only rejection of claim 38 is its rejection under 35 U.S.C. 102(a) as being anticipated by Doench et al. In addition to claims 32 and 38, claim 28 was not rejected under 35 U.S.C. 103(a) as being unpatentable over Bentwich. The only rejection of claims 19-22 based upon prior art is the rejection under 35 U.S.C. 103(a) as being unpatentable over Bentwich.

***Summary of the Amendment***

**Claims 12-18, 27, 28 and 31**

Claims 12-18, 27, 28, 31 and 34 have been canceled without prejudice.

**Claim 19**

Claim 19 has been amended to more clearly set forth the subject matter of the invention. As amended, claim 19 is now in independent form. Claim 19 has been amended to expressly recite method steps previously referred to by its dependency to claim 12. In addition, claim 19 as amended states the features of a microRNA according to the claim.

The preamble of claim 19 has been amended to refer to

A system for identifying a microRNA sequence that targets a  
selected mRNA sequence

In addition, the preamble as amended states that the microRNA is:

a nucleic acid molecule that is 17-25 nucleotides, has a sequence  
with complementarity to the selected mRNA sequence that is  
indicative of a microRNA to a microRNA recognition element  
and has a sequence that when paired with the selected mRNA  
sequence has free energy of -20 kcal/mole or less.

Claim 19 has also been amended clarify the “processor” component of the claimed system as one that is

programmed with instructions for performing a method of  
identifying a microRNA sequence that targets a selected mRNA  
sequence

Four steps of the method are set forth. The first step relates to the step by which the system received information regarding the selected mRNA sequence to be targeted. After the selected mRNA sequence is identified, an oligonucleotide is generated which has specific features, i.e. a sequence that is 17-25 nucleotides with complementarity to the selected mRNA sequence that is indicative of a microRNA to a microRNA recognition element. The amount of free energy of a pairing of the oligonucleotide sequence and the selected mRNA sequence is calculated. The claim states that

free energy of -20 kcal/mole or less identifies the pairing as having free energy of a pairing of a microRNA and a microRNA recognition element.

Finally, claim 19 as amended recites that an oligonucleotide sequence is identified that has 1) 17-25 nucleotides; 2) a sequence that has a degree of complementarity to the selected mRNA sequence that is indicative of a microRNA-recognition element for a microRNA and 2) a sequence that when paired with the selected mRNA sequence has free energy calculated to be -20 kcal/mole or less. Such an oligonucleotide sequence that is identified with these features is identified as a microRNA sequence of a microRNA that targets a selected mRNA sequence.

Support for claim 19 as amended is found throughout the specification such as on page 6-18, particularly for example pages 12, 16 and 17, and the claims as filed. No new matter has been added.

#### **Claim 22**

Similar to claim 19, claim 22 has been amended to more clearly set forth the subject matter of the invention. As amended, claim 22 is now in independent form. Claim 22 has been amended to expressly recite method steps previously referred to by its dependency to claim 12. In addition, claim 22 as amended states the features of a microRNA according to the claim.

The preamble of claim 22 has been amended in several respects. As amended, the preamble refers to refer to “a non-transitory computer readable medium” and additionally to delete a typographical error which was the basis of an objection to the claim. Further, the computer program is stated to be for use on a computer system

for identifying a microRNA sequence of a microRNA that targets a selected mRNA sequence.

The preamble of claim 22 as amended states that the microRNA is:

a nucleic acid molecule that is 17-25 nucleotides, has a sequence with complementarity to the selected mRNA sequence that is indicative of a microRNA to a microRNA recognition element and has a sequence that when paired with the selected mRNA sequence has free energy of -20 kcal/mole or less.

and that

the program comprises instructions for performing a method of identifying a microRNA sequence of a microRNA that targets a selected mRNA sequence.

Similar to claim 19 as amended, four steps of the method are set forth in claim 22. The first step relates to identifying the selected mRNA sequence to be targeted. After the selected mRNA sequence is identified, an oligonucleotide is generated which has specific features, i.e. a sequence that is 17-25 nucleotides with complementarity to the selected mRNA sequence that is indicative of a microRNA to a microRNA recognition element. The amount of free energy of a pairing of the oligonucleotide sequence and the selected mRNA sequence is calculated. The claim states that

free energy of -20 kcal/mole or less identifies the pairing as having free energy of a pairing of a microRNA and a microRNA recognition element.

Claim 22 as amended recites that an oligonucleotide sequence is identified that has 1) 17-25 nucleotides; 2) a sequence that has a degree of complementarity to the selected mRNA sequence that is indicative of a microRNA-recognition element for a microRNA and 2) a sequence that when paired with the selected mRNA sequence has free energy calculated to be -20 kcal/mole or less. Such an oligonucleotide sequence that is identified with these features is identified as a microRNA sequence of a microRNA that targets a selected mRNA sequence.

Support for claim 22 as amended is found throughout the specification such as on page 6-18, particularly for example pages 12, 16 and 17, and the claims as filed. No new matter has been added.

### **Claim 29**

Claim 29 refers to a method of preparing a microRNA comprising confirming the inhibitory activity of "a microRNA candidate oligonucleotide" by contacting it with cells and determining mRNA expression. The claim refers to the method by which the microRNA candidate oligonucleotide was produced.

Claim 29 has been amended to more clearly set forth the subject matter of the invention. As amended, claim 29 expressly sets forth the features of the “microRNA candidate oligonucleotide”, i.e. a 17-25 nucleotide nucleic acid molecule that

has a sequence with complementarity to the selected mRNA sequence that is indicative of a microRNA to a microRNA recognition element, and has a sequence that when paired with the selected mRNA sequence has free energy of -20 kcal/mole or less.

As amended, the steps performed to confirm that the microRNA candidate oligonucleotide inhibits expression of mRNA that comprises the selected mRNA sequence are presented more clearly. As amended it is clear that the steps involve contacting the microRNA candidate oligonucleotide with a cell that contains the mRNA and determining the expression of the mRNA. As amended, the claim states that a reduction in expression of the mRNA in the cells

indicates that the microRNA candidate is a microRNA that targets the selected mRNA sequence.

Claim 29 has also been amended throughout to eliminate confusing language. As amended, the steps setting forth the method by which the microRNA candidate oligonucleotide was produced have been clarified. The steps set forth include identifying the selected mRNA to be targeted and generating an oligonucleotide sequence having the size of a micro RNA and a sequence that has complementarity “indicative of a microRNA to a microRNA recognition element”. In addition, claim 29 has been amended to recite the step that that the amount of free energy is determined for a pairing of the selected mRNA sequence and the generated oligonucleotide sequence that that meets the complementarity criteria in order to determine if the amount free energy of the pairing is -20 kcal/mole or less. A nucleotide molecule is synthesized which comprises the 17-25 nucleotide sequence which meets the complementarity criteria and which when paired with the selected mRNA sequence has a free energy of -20 kcal/mole or less.

As amended, claim 29 clearly states that a microRNA candidate oligonucleotide has a sequence with specific features relating to size, the degree of its complementarity to the selected mRNA sequence and the amount of free energy when paired with the selected mRNA sequence. As amended, claim 29 clearly states that the microRNA candidate oligonucleotide is prepared by a method that includes determining that the amount of free energy when paired with the selected mRNA sequence is -20 kcal/mole or less.

Support for claim 29 as amended is found throughout the specification such as on page 6-18, particularly for example pages 12, 15-17, and the claims as filed. No new matter has been added.

#### **Claim 32**

As noted above, the only rejection of claim 32 was under 35 USC 101; claim 32 was free of the prior art. Claim 32 has been amended to make it an independent claim comprising all subject matter previously included by its dependency. In addition, the step of synthesizing a nucleic acid molecule, having a nucleotide sequence that is the microRNA oligonucleotide sequence. As amended, the claim recites a transformation of matter.

Support for claim 329 as amended is in the claims as filed. No new matter has been added.

#### **Claims 33-34**

Claim 33 has been amended to incorporate the subject matter of claim 34 and to more clearly set forth the subject matter of the invention. Claim 34 has been canceled as being redundant in view the amendment to claim 33.

To clarify the claim, the preamble has been amended to refer to “[a] microRNA that targets a selected mRNA sequence”. In addition, the preamble as amended states that the microRNA is:

a nucleic acid molecule that is 17-25 nucleotides, has a sequence with complementarity to the selected mRNA sequence that is indicative of a microRNA to a microRNA recognition element and has a sequence that when paired with the selected mRNA sequence has free energy of -20 kcal/mole or less.

The language in step II) which refers to the step of generating a oligonucleotide sequence has been amended to improve readability by placing the references to the degree of complementarity of the oligonucleotide sequence to the selected mRNA sequence in closer proximity to each other. Moreover, the term “microRNA oligonucleotide sequence” has been revised to read “oligonucleotide sequence” to avoid confusion.

Step III has been similarly amended. In addition, step III, which refers to “determining free energy” has been amended to add language from the specification in an effort to more clearly set forth the subject matter. As noted above, the subject matter of claim 34 has been incorporated into claim 33. Accordingly, as amended, claim 33 indicates that the threshold amount of free energy recited in the claim is “-20\_kcal/mole or less”.

Step IV, which is the step that refers to “synthesizing a nucleic acid molecule” has been amended to more clearly set forth that the nucleic acid molecule comprises an oligonucleotide sequence which has 17-25 nucleotides and a degree of complementarity with the selected mRNA sequence that is indicative of a microRNA for a microRNA-response element

that has a sequence that pairs with selected mRNA sequence with  
a free energy determined to be -20 kcal/mole or less.

Step IV states that the nucleic acid molecule which is synthesized comprises a sequence having the three specific features, i.e. 1) 17-25 nucleotides, 2) a sequence whose complementarity with the selected mRNA sequence is indicative of a microRNA for a microRNA-response element, and 3) a sequence that when paired with the selected mRNA sequence forms a pairing with a free energy that has been **determined** to be -20 kcal/mole or less.

Support for claims 33 as amended is found throughout the specification such as on page 6-18, particularly for example page 12, and the claims as filed. No new matter has been added.

### **Claim 35**

Claim 35 has been amended to more clearly recite that nucleic acid molecule that is synthesized is a microRNA oligonucleotide sequence (i.e. per step II of claim 33, a 17-25

nucleotide sequence that has the complementarity indicative of a microRNA for a microRNA recognition element)

that has a sequence that when paired with the selected mRNA sequence has free energy determined to be -30 kcal/mole or less.

As amended, claim 35 states that the nucleic acid molecule synthesized in accordance with the method of claim 35 has had the free energy of a pairing of its sequence paired and the selected mRNA sequence determined to be -30 kcal/mole or less.

Support for claim 35 as amended is found throughout the specification such as on page 6-18, particularly for example page 12, and the claims as filed. No new matter has been added.

#### **Claim 38**

Claim 38 has been amended to more clearly recite that the testing step results show an “inhibition” of mRNA expression.

Support for the amendment is found throughout the specification and the claims as filed. No new matter has been added.

#### **New Claims 39-48**

New claims 39-42 correspond to claim 38 except new claims 39-42 recites that the testing step “results in inhibition of the expression of the mRNA by” at least 20%, at least 40%, at least 60%, and at least 80%, respectively.

New claims 43-48 correspond to claims 35, 36 and new claims 43-48, respectively, except new claims 43-48 are dependent on claim 29.

Support for new claims 39-48 is found throughout the specification such as on page 6-18, particularly for example pages 12, 15 and 16, and the claims as filed. No new matter has been added.

#### ***Objections to Claims***

Objections have been made to claims 14-19, 22, 34 and 35.



Objections have been made to claims 14, 15, 34 and 35 under 37 CFR 1.75(c), as being in improper dependent form for failing to further limit the subject matter of a previous claim. It is asserted in the Official Action on page 2 that in claim 14, the limitation

wherein a free energy determination of -20 kcal/mole or less indicates that said mRNA sequence is a microRNA-recognition element for microRNA

does not further limit claim 12 because it does not contain any active method step for determining free energy. Further, it is asserted that

the claim language is open and does not indicate that binding pairs with a free energy of greater than -20 kcal/mol are not indicative of a miRNA-MRE relationship.

(Official Action, page 3) Claims 15, 34 and 35 are asserted to have a similar deficiency. The Office states that the “open” language of claims 34 and 35 does not

exclude binding pairs with a free energy of greater than -20 kcal/mol from the genus of binding pairs in a miRNA-MRE relationship required by the claims.

The objections to claims 16-19 relates to an obvious typographical error in which each of the claims improperly uses the plural form in its reference to “claims 12”. Similarly, the objection to claim 22 concerns an obvious typographical error in the preamble which superfluously contains the word “that”, stating: “A computer system that for . . .”

Claims 14-18 and 34, have been canceled and the objections with respect to those claims are moot.

Claim 19 and 22 have been amended to correct the typographical errors and the bases for their objections are moot.

Claim 35 has been amended to more clearly state that the nucleic acid which is synthesized

that has a sequence that when paired with the selected mRNA sequence has free energy determined to be -30 kcal/mole or less.

Claim 35 as amended excludes methods of preparing microRNA having sequences which when paired with the target mRNA have a free energy of great than -30 kcal/mole. For nucleic acid

molecules synthesized according to claim 35, the free energy of the pairing has been determined by be -30 kcal/mole or less. As amended, claim 35 further limits claim 33. As amended, the objection to claim 35 has been obviated.

Applicants respectfully request that the objection to claims 14-19, 22, 34 and 35 as applied to claims 19, 22 and 35 be withdrawn.

***Claim Rejections - 35 U.S.C. §112***

Claims 12-22, 27 and 28 have been rejected under 35 U.S.C. 112, second paragraph, as failing to particularly point out and distinctly claim subject matter which Applicant regards as the invention. Claim 12, from which each of claims 13-22, 27 and 28 ultimately depend, contained a typographical error in the section under heading b) which was asserted to render the claims indefinite. Moreover, claim 16 was asserted to not define the metes and bounds of the claim properly.

Claims 12-18, 27 and 28 have been canceled and the rejection is as applied to those claims is moot.

Claim 19 and 22, which were each formerly dependent on claim 12 have each been rewritten in independent form. In sections of claims 19 and 22, respectively, corresponding to heading b) of claim 12, the language has been corrected to eliminate the error at issue in the Official Action.

Claims 20 and 21, which are dependent on claim 19 and thereby formerly dependent on claim 12 no longer depend on claim 12 in view of the amendment of claim 19. As amended, claim 19 does not contain the error that was in claim 12 which formed the basis of the rejection. Accordingly, claims 20 and 21 are clear and definite.

Claims 19-22 are in compliance with the requirements of the second paragraph of 35 U.S.C. 112. Applicants respectfully request that the rejection of claims 12-22, 27 and 28 under 35 U.S.C. 112, second paragraph, as failing to particularly point out and distinctly claim subject matter which Applicant regards as the invention as applied to claims 19-22 be withdrawn.

***Claim Rejections - 35 U.S.C. §101***

Claims 12-18, 22, 31 and 32 have been held to claim an abstract idea and therefore rejected under 35 U.S.C. 101 as being ineligible subject matter.

With regard to claims 12-18, 31 and 32, it is asserted that the subject matter as claimed is not statutory subject matter.

With regard to claim 22, it is asserted that the claimed computer program does not exclude embodiments which constitute non-statutory subject matter

Claims 12-18 and 31 have been canceled and the rejection is as applied to those claims is moot.

Claim 22 has been amended to recite that the claims computer program is “embodied on a **non-transitory** computer readable medium”. As amended, claim 22 refers to statutory subject matter only.

Claim 32 as amended, contains an added step of synthesizing “a nucleic acid molecule”. As amended, claim 32 refers to statutory subject matter.

Claims 22 and 32 as amended are directed to eligible subject matter. Applicants respectfully request that the rejection of claims 12-18, 22, 31 and 32 under 35 U.S.C. 101 as being ineligible subject matter, as applied to claim 22 and 32, be withdrawn.

***Claim Rejections - 35 U.S.C. §102***

**Doench et al.**

Claims 12-18, 27-30 and 33-38 have been rejected under 35 U.S.C. 102(a) as being anticipated by Doench et al.

Claims 12-18, 27 and 28 have been canceled and the rejection is as applied to those claims is moot.

Claims 29, 30, 33 and 35-38 remain pending, claims 29, 33, 35 and 38 as amended.

Doench discloses oligonucleotides which are asserted to suppress mRNA translation.

Claims 29, to which claim 30 is dependent, and claim 33, to which claims 35-38 are dependent, have each been amended to recite that the microRNA is

a nucleic acid molecule that is 17-25 nucleotides, has a sequence with complementarity to the selected mRNA sequence that is

indicative of a microRNA to a microRNA recognition element  
and has a sequence that when paired with the selected mRNA  
sequence has free energy of -20 kcal/mole or less,

and that the synthesized nucleic acid molecule” comprises an oligonucleotide sequence which  
has 17-25 nucleotides and a degree of complementarity with the selected mRNA sequence that  
is indicative of a microRNA for a microRNA-response element as specified within the claim  
and

has a sequence that pairs with selected mRNA sequence with a  
free energy **determined** to be -20 kcal/mole or less.

Doench does not disclose methods of preparing a microRNA which comprise identifying  
an oligonucleotide sequence that have 17-25 nucleotides, a sequence with complementarity to  
the target sequence that is indicative of a microRNA:MRE as defined in the claims as amended,  
and a sequence which pairs with the selected sequence with free energy of -20 kcal/mole or less.  
The free energies reported in Doench are greater than -20 kcal/mole. Note, as a negative  
number, less than -20 kcal/mole includes for example -21 kcal/mole, -22 kcal/mole ... -30  
kcal/mole etc. Doench observed differences in importance of free energy of pairing between free  
energy of pairings of the proximal portion compared to between free energy of pairings of the  
distal portion but does not teach the claimed method or suggest the invention.

The Official Action notes with respect to claims 13, 33 and 34 that

The claim language is open and does not exclude mRNA regions  
with binding energies of greater than -10 kcal/mol from being  
MREs for the miRNA. Thus mRNA targets with  
complementarity to the miRNA that provides a binding energy of  
greater than -10 kcal/mol are not excluded by the claim from  
being a micro-RNA recognition element for the recited miRNAs

(Official Action, page 9) As amended, claims 29 and 33 state that properties of a nucleic acid  
molecule that is a microRNA include 1) 17-25 nucleotides, 2) a sequence that has a degree of

complementarity to the selected mRNA sequence that is  
indicative of a microRNA to a microRNA recognition element

and a sequence that pairs with the selected mRNA sequence with “a free energy of -20  
kcal/mole or less”. Nucleic acid molecules synthesized according to the methods of claim 29

and 33 necessarily not only have these properties but have been prepared based upon such sequences being determined to form pairs with the selected mRNA sequence with “a free energy of -20 kcal/mole or less”. Doench did not do this.

With respect to claims 14, 15, 34 and 35, the Official Action states that these claims are included in the rejection because as used in claims 14 and 35, the phrase

wherein a free energy determination of -20 kcal/mole or less indicates that said mRNA sequence is a microRNA-recognition element for microRNA

(Official Action, page 9) and as used in claims 15 and 35, the phrase

wherein a free energy determination of -30 kcal/mole or less indicates that said mRNA sequence is a microRNA-recognition element for microRNA

carry no patentable weight since they does not limit the claims in any way. That is, because claims 14, 15, 34 and 35 require

no step of measuring free energy of binding, so the language regarding binding energy determination carries no weight. Moreover, while the claim indicates that mRNA regions with binding energies of -20 kcal/mole or less for a given miRNA are considered to be MREs for that miRNA, it does not exclude mRNA regions with binding energies of greater than -20 kcal/mol from being MREs for the miRNA.

(Official Action, page 9)

As amended, claim 33 included the step of determining free energy of the pairings of oligonucleotides and the selected RNA sequence. Further the amendments of claims 29 and 31 make clear that free energy of -20 kcal/mole or less for pairings of the microRNA sequence and selected mRNA sequence are considered to be MREs for that miRNA, and that free energy of greater than -20 kcal/mole for pairings of the microRNA sequence and selected mRNA sequence are not considered to be MREs for that miRNA and are excluded from the claimed methods.

As amended, claims 29, 30, 33 and 35-38 are not anticipated by Doench. Applicants respectfully request that the rejection of claims 12-18, 27-30 and 33-38 under 35 U.S.C. 102(a) as being anticipated by Doench et al., as applied to claims 29, 30, 33 and 35-38 be withdrawn.

**Zeng et al.**

Claims 12, 14-18, 27 and 28-30 have been rejected under 35 U.S.C. 102(b) as being anticipated by Zeng et al.

Claims 12, 14-18, 27 and 28 have been canceled and the rejection is as applied to those claims is moot.

Claims 29 and 30 remain pending, claim 29 has amended.

Zeng discloses oligonucleotides which are asserted to suppress mRNA translation. Zeng does not disclose a method which includes the step of determining free energy. accordingly indicated to have been included in that rejection for the same reason

Claims 29, to which claim 30 is dependent, has been amended to recite that the microRNA is

a nucleic acid molecule that is 17-25 nucleotides, has a sequence with complementarity to the selected mRNA sequence that is indicative of a microRNA to a microRNA recognition element and has a sequence that when paired with the selected mRNA sequence has free energy of -20 kcal/mole or less,

and that the synthesized nucleic acid molecule” comprises an oligonucleotide sequence which has 17-25 nucleotides and a degree of complementarity with the selected mRNA sequence that is indicative of a microRNA for a microRNA-response element as specified within the claim and

has a sequence that pairs with selected mRNA sequence with a free energy **determined** to be -20 kcal/mole or less.

As amended, claims 29 and 30 specifically refer to subject matter which is neither disclosed nor suggested in Zeng.

As amended, claims 29 and 30 are not anticipated by Zeng. Applicants respectfully request that the rejection of claims 12-18 and 27-30 under 35 U.S.C. 102(b) as being anticipated by Zeng et al., as applied to claims 29 and 30 be withdrawn.

**Bentwich et al. in view of evidence of Tinoco.**

Claims 12-17 and 31 have been rejected under 35 U.S.C. 102(e) as being anticipated by Bentwich et al. in view of evidence in Tinoco.

Claims 12-17 and 31 have been canceled and the rejection is moot. Applicants respectfully request that the rejection of claims 12-17 and 31 under 35 U.S.C. 102(e) as being anticipated by Bentwich et al. in view of evidence in Tinoco be withdrawn.

***Claim Rejections - 35 U.S.C. §103***

**Bentwich**

Claims 12-22, 27, 29-31 and 33-37 have been rejected under 35 U.S.C. 103(a) as being unpatentable over.

Claims 12-18, 27, 28, 31 and 34 have been canceled and the rejection as applied to those claims is moot.

Claims 19-22, 29, 30, 33 and 35-37 remain pending, claims 19, 22, 29, 33 and 35 as amended.

Bentwich discloses group of bioinformatically detectable novel genes identified as “genomic address messenger” that Bentwich indicated are believed to be related to the microRNA. In the rejection of claims 12-17 and 31 as anticipated by Bentwich, two sequences are disclosed which are asserted to meet the disclosed complementarity indicative of a microRNA for a microRNA recognition element. Applicants respectfully note that the first example included in the Official Action does not because while the binding site (SEQ ID NO:362,996) includes an mRNA region corresponding to the microRNA proximal region of the microRNA (SEQ ID NO:20,604) that is completely complementary to the microRNA proximal region, and includes an mRNA region corresponding to the microRNA loop region of the microRNA that either forms an mRNA loop of 2-5 non-paired nucleotides of mRNA when

the microRNA loop region of the microRNA is 0, it does not includes an mRNA region corresponding to the microRNA distal region of the microRNA in compliance with the required features. That is the mRNA region corresponding to the microRNA distal region of the microRNA is not completely complementary to at least 7 contiguous nucleotides of the microRNA distal region of the microRNA including the 5' end of the microRNA distal region nor does it contain (A) mismatches of 1-4 contiguous nucleotides and (B) matches of at least 5 nucleotides to a contiguous nucleotide sequence of the microRNA distal region of the microRNA, including the 5' end of the microRNA distal region. It contains mismatches of 3 contiguous nucleotides but it does not contain at least 5 nucleotides to a contiguous nucleotide sequence of the microRNA distal region of the microRNA, including the 5' end of the microRNA distal region. The nucleotide pair at the position corresponding to the fourth nucleotide continuous with the 5' end of the microRNA distal region is a mismatch of G at the position fourth from the 5' end of the microRNA distal region and T on the corresponding nucleotide of the binding partner. The second example comprising the binding site (SEQ ID NO:69,717) includes an mRNA region corresponding to the microRNA proximal region of the microRNA (SEQ ID NO:20,604) that is in compliance with the rules. However, Bentwich neither taught nor suggested that a microRNA is

a nucleic acid molecule that is 17-25 nucleotides, has a sequence with complementarity to the selected mRNA sequence that is indicative of a microRNA to a microRNA recognition element and has a sequence that when paired with the selected mRNA sequence has free energy of -20 kcal/mole or less

as set forth in the claims nor does Bentwich teach or suggest a method of preparing microRNA that includes the step of has calculating or otherwise determining

free energy of a pairing of the oligonucleotide sequence and the selected mRNA sequence to identify the pairing as having free energy of a pairing of a microRNA and a microRNA recognition element, wherein a free energy determination of -20 kcal/mole or less indicates that said selected mRNA sequence is a microRNA-recognition element for a microRNA having the oligonucleotide sequence.



Bentwich does not disclose that nucleic acid molecules are synthesized that are 17-25 nucleotides, have a sequence with complementarity to the selected mRNA sequence that is indicative of a microRNA to a microRNA recognition element as set forth in the claims and have

a sequence that when paired with the selected mRNA sequence  
was determined to have free energy of -20 kcal/mole or less

The Official Action states that with regard to claim 33, it would have been obvious to one skilled in the art to synthesize and test microRNAs against the selected mRNA sequence. The teachings of Bentwich are silent with respect to the determining of free energy of the of the oligonucleotide sequence and the selected mRNA sequence, much less that the threshold free energy determination of -20 kcal/mole or less as in claim 33 as amended (formerly in claim 34) indicates that said selected mRNA sequence is a microRNA-recognition element for a microRNA having the oligonucleotide sequence, or the threshold free energy determination of -30 kcal/mole or less as in claim 35 as amended. The Official Action states

Regarding claims 34 and 35, and the recitation that a free energy determination of -20 or -30 kcal/mol indicates that the target mRNA sequence is a MRE for the generated miRNA, it is reiterated that this recitation does not further limit the claims, and so claims 34 and 35 are obvious for the same reasons applied to claim 33.

(Official Action, page 16). As discussed above, the claims as amended recite that the free energy component is a claim limitation which excludes subject matter greater than the stated threshold. Further, claims 29, 33 and 35 as amended, and new claim 43 specifically recite that the nucleic acid molecules synthesized have been determined to be within the limitation stated in the claim.

Bentwich does not disclose using free energy in combination with complementarity to prepare microRNA or provide computer tools for doing the same. Bentwich refers to free energy determinations in the bioinformatic search tool used to identify potentially hairpin sequences of RNA molecules which may be precursors to microRNA. The free energy

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determination described in Bentwich was for a completely different purpose, i.e. a way of scanning linear sequences to identify possible sites which can form hairpins, not between two molecules. The claimed invention relates to preparing microRNA by identifying a target mRNA sequence and generating an oligonucleotide sequences that has a sequence with both a defined complementarity to the target and a define free energy for pairings of the oligonucleotide sequence and target sequence.

The claimed invention is not disclosed in Bentwich, nor is it suggested. One skilled in the art would not consider the claimed invention prima facie obvious in view of Bentwich. Applicants respectfully request that the rejection of claims 12-22, 27, 29-31 and 33-37 under 35 U.S.C. 103(a) as being unpatentable over Bentwich, as applied to claims 19-22, 29, 30 and 33-37 be withdrawn.

***Conclusion***

Claims 19-22, 29, 30, 32, 33 and 35-48 are in condition for allowance. A notice of allowance is earnestly solicited. Applicants invite the Examiner to contact the undersigned at 610.640.7855 to clarify any unresolved issues raised by this response.

The Commissioner is hereby authorized to charge any deficiencies of fees and credit of any overpayments to Deposit Account No. 50-0436.

Respectfully submitted,

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